RELATION OF THE TRUE NUCLEOLUS TO THE LININ NETWORK IN THE GROWTH PERIOD OF PSELLIODES CINCTUS.[1]

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(THIRTY FIGURES; TWO PLATES.)

INTRODUCTION.

The purpose of the present line of investigation is to follow the nucleolus in its relation to the other parts of the cell. Except for the extended review and observations of Montgomery ('98), the work on the true nucleolus is scattered in small bits throughout the cytological literature from the first observations of Frontana (1781) to the present time. Especially since the discovery of the sex-chromosomes has the literature on this subject been of a fragmentary character, consisting usually of a few records here and there in papers dealing with other subjects.

The work on the true nucleolus has been greatly retarded by the similarity of the staining reactions of different nuclear bodies and also by the confusion of terms which various observers have used to indicate these nuclear bodies which have an affinity for acid tar-colors and other plasma stains. Ogata ('83) was the first to use the term "plasmosome" with reference to the true nucleolus. The term "paranuclein" of O. Hertwig ('92) and "pyrenin" of Schwartz ('87) are ofttimes erroneously applied to the plasmosome itself, rather than to the substance of the same. Eisen ('98) used the term "linoplast," since he insists that the function of this body is to supply and nourish the linin network during mitosis. To prevent further confusion, I shall use the older and more familiar term, "nucleolus." The term "nucleolus" however carries with it no definite meaning and thus should not be used without a modifying adjective, either expressed or implied, as the cytological literature contains many errors and misconceptions on account of the lack of a clear dis-

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tinction between the various kinds of nucleoli. This difficulty renders it almost impossible to present a satisfactory review of the literature of the plasmosome, as so many writers discuss the "nucleoli" when even the context fails to indicate the kind of nucleoli under consideration. Carnoy ('85) finds four distinct kinds, namely, "nucléoles nucléiniens, nucléoles plasmatiques, nucléoles mixtes, and nucléoles noyaux." It seems advisable, however, to adhere closely to the well-known broader classification (chromatic and achromatic nucleoli) and always endeavor to make the distinction very clear.

Observations.[1]

It would be very desirable to have at hand a large number of species which show the points under consideration. As this is impossible at this time, I shall present the results based upon a study of *Pselliodes* (*milyas*) *cinctus* Fabr.

The testes of *Pselliodes cinctus* are composed of cylindrical but tapering follicles which are wound about in a very irregular form. Each follicle contains definite walled cysts, but on account of this winding shape it is very unusual to obtain even a longitudinal section which shows more than a half dozen cysts in the same follicle. This difficulty, however, is offset by the fact that the follicles show a regularity in the zonation of the various stages of development from one end to the other. Even though many stages of growth may be omitted, no cyst has been found containing cells which are in a more advanced stage than those further down the follicle. This makes it a comparatively easy matter to obtain the sequence of stages in development.

Since it is primarily beyond the scope of this paper to deal with the chromatic parts of the cell, except so far as related directly to the development of the achromatic parts of the nucleolus, the figures include only the stages of the growth period between the synaptic stage and the first spermatocyte division. Incidentally, however, observations were made upon the stages of spermatogenesis both preceding the appearance and following the disappearance of the plasmosome material. The periods in

[1] While working on the sex-chromosomes in the Reduviidæ, Dr. F. Payne made observations which enabled him to suggest the present line of research.

maturation prior to the synaptic stage correspond in general to those of *Oncopeltus* as given by Wilson ('12). It was further noted, in agreement with the observations of Payne ('12), that the oögonial number of chromosomes is thirty, the spermatogonial twenty-eight, and the first spermatocyte sixteen. The metaphase plate of the second maturation division shows twelve chromosomes arranged in a more or less uniform circle surrounding four (three small and one large) sex-chromosomes.

A. Origin, Development and Fate of the Nucleolus.

The contraction stage presented in Fig. 1 is of considerable duration. As this figure shows, the chromatin threads are thick and stain intensely. The threads even though in a contracted condition, exhibit a certain degree of polarization, many of the fibers terminating in the vicinity of the nucleolus, but seldom if ever coming in contact with it.

Embedded in the periphery of this mass of chromatin threads lies the large, compact, well-defined nucleolus surrounded by a vacuole-like space as described and illustrated by Wilson ('12) in *Largus* and other forms. As he says of *Largus* (Figs. 78 to 80), "no definite wall to the vacuole can be seen, but the chromatin threads are often seen encircling its outer limit as if lying upon a definite substratum." This noteworthy feature is characteristic of all the preceding stages back to the telophase of the last spermatogonial division. However, in the earlier stages the body is somewhat smaller and more spherical in form. This slight increase in size and the change of shape suggests a certain degree of activity prior to the stage represented in Fig. 1. After this period, however, the growth is so rapid that seldom can two adjacent cysts be found which show the same stage of nucleolar development.

Under ordinary staining conditions the nucleolus shows no differentiation in the structure until after the nuclear wall has formed (Fig. 15). In fact the condition was so universal that up to this stage the nucleolar body was first considered solely chromatic in nature. Later observations, however, revealed the fact that *the nucleolus is not a homogeneous body but composed of both chromatic and achromatic material.* This conclusion is based

upon a study of the development of this structure and its reaction to stains. The earliest possible differentiation that can be observed is found immediately following the synaptic stage (Figs. 5 and 6). The nucleolus at this time is of a more or less irregular shape, and sometimes certain parts of it take the stain less intensely. The more deeply staining areas give the appearance of irregular granular masses. Although later development shows that these masses are the sex-chromosomes in a diffused condition, they are in this early stage poorly defined (Figs. 12 and 13).

As the cell continues to grow, a gradual growth in the nucleolus is likewise noted. The distinction between the chromatin and the achromatin becomes more noticeable as the chromatin aggregations gradually condense. The condensation of this chromatin material causes certain areas of the nucleolus to become clearer, or to take the plasma stain in case a counter stain is used. Many instances are noted in which one end of a chromatin body is much more compact than the other (Figs. 13 and 16), in which case the corresponding achromatic part of the nucleolus is much more homogeneous.

Another noteworthy feature, characteristic of these stages in the early life of the nucleolus, is the irregular contour. In practically every nucleolus the more granular and loose in texture the chromatic areas are, the more irregular is the whole body. For example, Fig. 14 shows various evaginations of the plasma material in which are found chromatin-granules either in a loose (*d*) or compact (*a*) condition. After the chromatin granules are condensed into four compact bodies, the sex-chromosomes, the whole mass presents a more globular appearance (Figs. 17, 18, 19 and 20). Fig. 18 is a typical figure of this stage. The condition shown in Fig. 17 is seldom observed, while only three or four cells were found which show four clear-cut chromosomes in the same plane (Fig. 20). There is nothing problematical regarding this change to a uniform spherical shape, as the withdrawal of the chromatin granules leaves the achromatic portion of the nucleolus less dense and thus gives it an opportunity to assume a more globular form, in accordance with the general laws of fluid action. In the previous stages the

amount of plasma substance is so small in comparison with the embedded chromatin, that the latter acts as the governing factor in determining the shape of the whole mass; but by the time the stage under consideration (Figs. 17 to 20) is reached, the achromatic part of the nucleolus is sufficiently large to be only slightly affected by the presence of the embedded bodies. However, in cases where the nucleolus has a slight irregular contour, the same can usually be accounted for by the presence of one or more of the sex-chromosomes near the periphery.

The nucleus now has a period of very rapid growth accompanied by noticeable extra-nucleolar changes. In the earlier stages the nucleus presents a somewhat uniform fibrillar but granular appearance, though much darker near the nuclear membrane than in the central part. The clear area around the nucleolus increases in size and, in the middle growth period, shows no traces of linin. As the cell continues to grow the linin becomes more compact, first near the nuclear wall and then nearer and nearer the nucleolus (Figs. 16 to 20). Numerous instances may be seen where entanglements of linin are scattered indiscriminately throughout the nucleus even into the vacuole-like space.

At a little later stage this whole complex mass between the nucleolus and nuclear wall, presents the appearance of innumerable anastomosing threads of a fibrillar substance, in which are embedded minute granules. These fibers continue to elongate until they come in contact and ultimately fuse with the true nucleolar portion of the nucleolus. This fusion, together with a continued growth on the part of both the true nucleolus and the surrounding linin, causes the two to become continuous and to assume a similar appearance (Figs. 22 and 23). Prior to this time the achromatic part of the nucleolus has presented a typical homogeneous appearance, but as more granular fibers come in contact and fuse with it, it assumes a more granular appearance (Figs. 21–25). The achromatic part of the nucleolus now greatly resembles the granular appearance shown in the stages prior to the individualizing of the sex-chromosomes. This condition suggests that chromatin granules have moved along the linin and entered the true nucleolus. *This indicates that the plasmo-*

some is linin or closely related in composition to it. As the linin and the achromatic body becomes continuous the latter is pulled into various shapes and becomes practically indistinguishable from the linin (Fig. 25). While these changes are taking place, the peripheral areas of linin become more conspicuous—the larger number of these aggregations being either near or in contact with the nuclear membrane. In order to form the chromosomes the linin collects, leaving wide intervening spaces almost clear (Figs. 23–26). Usually in the formation of these aggregations, strands of linin connecting the centralized mass (the former nucleolus) and the nuclear membrane remain in contact on one side longer than on the other. Whether or not this action is associated with the movement of the central mass to the vicinity of the nuclear wall in early prophase, we can only surmise (Figs. 25 and 26). In the meantime the achromatic parts of the nucleolus and the linin network, become more and more indistinguishable from one another.

As the aggregations of chromatin and linin become more compact, in the formation of the bivalent chromosomes of the first spermatocyte division (Figs. 28 to 30), masses and strands of achromatin may be observed attached to the chromosomes and floating in the cell sap (Fig. 27). This same condition is figured by Stevens ('11) in the spermatocyte cells of the guinea pig. She notes that "in early prophase the chromatin appears as though gathering together about definite centers along the spireme, leaving the linin threads between." However, the chromosomes in the material under consideration, are so far apart that the strands of linin are usually broken between them. By this time, no discrimination can be made between the achromatic part of the former nucleolus and the linin, except in a few cases where large collections of achromatic material is indicative of the former (Fig. 28).

Discussion.

It would be next to impossible to draw conclusions of general application from the above observations, since they are based upon only one species. It seems advisable, however, to call attention to some of the important facts regarding the origin,

development, fate, and function of the nucleolus of *Pselliodes*, and to compare these observations with those of a few other workers who have incidentally noted facts along the same line.

Origin.—Since the chromatin and achromatin of the nucleolus are indistinguishable in the early growth period, it is impossible to follow the achromatic material back to its first appearance. My observations, however, clearly show that its origin is in some way related to the chromatin. This suggestion does not corroborate the views of Montgomery and many earlier workers, but finds support in the observations of a large number of later investigators. The theory of the extra nuclear origin of the achromatic nucleolus, as set forth by Korschelt ('89) and strongly supported by Montgomery ('98) readily loses its significance, at least in general application, when the life history of the nucleolus of *Pselliodes* and similar forms are understood. The only proof set forth in support of this view is the fact that such nucleoli first appear in contact with the nuclear membrane. In many forms, the true nucleolus not only originates independent of, but completes its life history without being in any way associated with the nuclear membrane. Medes ('04), for example, notes that in the growth period of *Scutigera forceps* the chromatin accumulates, forming a karyosphere which seems to be of an achromatic nature, although containing the chromatin in granular form. The granules finally emerge leaving an achromatic mass, containing an accessory chromosome. The achromatic material now breaks into small rounded bodies, which soon become indistinguishable. The true nucleolus of *Philosamia cynthia* (Dederer, '07) also appears in a mass of chromatin entangled in the spireme threads of the last spermatogonial stage. In this and later stages, it is characteristically bipartite, one end of which usually stains darker, no doubt from the fact that it contains more chromatin granules. In its typical form, the idiochromosomes are attached around the middle of the double achromatic nucleolus in the form of a deep crescent-shaped band. This band later becomes shorter and broader until it loses contact with the nucleolus, which then disappears.

Although these citations will suffice to show that in many forms there is a certain uniformity in the early history of the

nucleolus, I wish to refer to the fact that many observers have found the sex-chromosomes either imbedded in, or associated with an achromatic nucleolus, as considered in detail in *Pselliodes*. Although the writers referred to below did not follow in detail the earlier history of the achromatic material many of their illustrations and descriptions suggest a uniformity. Payne ('09) finds the sex chromosomes of a number of Reduviidæ embedded in a plasmosome-like body as shown in *Pselliodes* (Figs. 17–19). He notes an achromatic nucleolus and a chromatic body (later the differential chromosomes) in the synaptic stage of *Prionidus cristatus*. After this stage the achromatic material forms around the chromatic body, and by fusion of the two bodies, a condition similar to figure 20 is assumed. Wilson ('05) figures a close relationship between the plasmosome and idiochromosomes of *Lygæus* and *Brochymena*. Boring ('07) notes a true nucleolus associated with an odd chromosome in *Aphrophora quadrangularis;* while in the blue rove beetle, *Staphylinus violaceus* (Stevens '08), and the earwig, *Anisolabis martima* (Randolph '08), this body is associated with a heterochromosome. Stevens ('08) notes that, "in the growth stage [of *Calliphora vomitoria*] the hetero-chromosomes are associated with a plasmosome as in many species of Coleoptera." Her Fig. 11 is suggestive of the condition found in the early history of the nucleolus of *Pselliodes*.

The direct relation which exists between the chromatin and the achromatin, clearly accounts for the erroneous theories of the earlier workers regarding the origin of the true and chromatin nucleolus. Montgomery ('98) states that according to his observations the true nucleolus is never derived from the chromatin. King ('08) notes that the achromatic body (in *Bufo lentiginosus*) is either embedded in the chromatin granules or surrounded by balls of chromatin attached to the surface, and further that "there is nothing to indicate that the chromatin in these structures (karyospheres) is derived from the plasmosome or vice versa." Ruzicka ('06) considers the true nucleolus as an intermediate stage between the chromatin and the linin. *My own observations have led me to conclude that in Pselliodes the true nucleolus is formed by the accumulation of linin about the sex-chromosomes.*

Development and Fate.—The observations on the development and disappearance of the plasmosome in *Pselliodes* seem to find agreement in many other forms. The observations of Medes ('04) on *Scutigera* and of Dederer ('07) on *Philosamia* are representative types of this agreement.

The latter part of the life history of the nucleolus, in which this body is again assuming a granular texture, is suggestive of that of *Culex* (Stevens '10), in which the achromatic body absorbs chromatin substance extruded from the spireme during the synizesis stage. Stevens bases her conclusion on the fact that the body presents a series of colorations in the growth period. She continues: "Whether the rejected material [from chromatin], visible in some cases, is waste material or substances which have some function connected with the growth stage of the germ cell, we can only surmise." No doubt, *Pselliodes* presents better material for a study of this point, as the foregoing observations clearly show that this chromatin is used in the formation of the first spermatocyte chromosomes (Figs. 26 to 30).

The later stages in the life of the nucleolus, in *Pselliodes*, lead to a suggestion as to its nature. It will be remembered that the linin becomes attached to various parts of this spherical body. The achromatic part then assumes a fibrillar but granular appearance, and becomes indistinguishable from the linin. These facts and observations, together with the recognized nature and functions of the linin, lead me to suggest that *the plasmosome and linin not only possess similar characters, but are one and the same material, the former being simply a globular mass of the latter.*

Function.—Since the achromatic part of the nucleolus of *Pselliodes* seems to be a modified form of the linin, we should expect a certain similarity in the functions of each. Further, since the function of the linin is to support the chromatin during the various stages of cell activity, we would attempt to ascribe a similar function to the plasmosome. A brief review of the preceding observations and illustrations clearly shows that we are not disappointed in the attempt. In *Pselliodes* the behavior of the sex-chromosomes and the achromatic material is identical with the corresponding action of the chromatin and linin. The

compact chromatin nucleolus of the early growth period takes up particles of achromatic material, and as the chromatic material assumes a less compact form, more achromatin (linin) is added, seemingly to act as a support to the chromatin aggregations. This growth continues until the sex-chromosomes are well differentiated and the true nucleolar body reaches its maximum size. *This behavior, as well as the method of breaking down of the achromatic material, indicates that the plasmosome has the same function that has been ascribed to the linin, namely, to support the chromatin material.*

B. Granules and Nuclear Membrane Formation.

In addition to the origin and early development of the nucleolus, the first thirteen drawings are intended to show the apparent relation that exists between certain granules and the formation of the nuclear membrane. The facts involved in this additional observation are not directly related to the problem in hand, but since they are presented in the same series of illustrations, a discussion of them does not seem out of place.

As was suggested in the preceding discussion, the chromatin fibers of the synaptic stage (Figs. 1 and 2) are thick and stain intensely. These threads, though in a contracted condition, possess a number of granular, knob-like enlargements, which stain more intensely than do the remaining parts of the thread. After the threads remain in this condition for some time, they elongate and decrease in thickness (Figs. 3, 4 and 6).

By virtue of this elongation, the chromatin mass becomes less compact and thus increases in size. At the stage represented in Fig. 4, the bead-like enlargements on the threads, though smaller and more compact, have become more defined, still retaining the stain. Thus they become somewhat individualized, while the part of the thread intervening between the beads takes the chromatin stain less intensely. This gives the thread the appearance of a number of granules (perhaps chromomeres) held in place by some substance quite dense in consistency. In fact, many chromatin fibers were found in this and subsequent stages, in which the contrast was so great between the deeply staining granular enlargements and the intervening spaces, that the

whole thread presented the appearance of a row of granular bodies with only here and there a fibrous connection (Figs. 7 and 8).

As the chromatin threads begin to elongate after the synaptic stage, the chromatic area as a whole begins gradually to stain less intensely until the stage represented in Fig. 7 is reached, when, in good preparations, nothing can be observed except the large nucleolus, surrounded by faint traces of fibers and a large number of granules. This gradual decrease in the staining capacity of the chromatin material rendered the tracing of the various transitional stages very difficult. Although the evidence was not entirely conclusive, the most careful observations suggested that the granules found in this and later stages were formerly a part of the chromatin threads and are distinctly chromatic in nature—perhaps being either a part of the true chromosomes or directly related to them.

Throughout the early growth period, no traces of a nuclear wall has been observed. In many and perhaps the majority of cells, the chromatin threads and granules seem to have a definite limiting space. However, among the same and in other cells, innumerable instances can be found in which the granules and granular rows are continuous far beyond what could be considered the nuclear area (Figs. 8 and 9), thus indicating the non-existence of a nuclear membrane. Flemming in the blood cells of Amphibians, Hertwig in the sperm-mother-cells of Nematodes, and other more recent workers have referred to the difficulty involved in the demonstration of the presence of a nuclear membrane in certain stages of cell growth.

The granules are now found scattered indiscriminately throughout the entire cell. The natural growth of the nucleolus and a simultaneous enlargement of the surrounding clear area, causes these granules to be crowded together between the nucleolus and the cell wall, forming a more or less incomplete circle around the nuclear area (Figs. 10 and 11). As more and more granules are added to the circle, visible strands of linin can be noted between them. The next stages (Figs. 11, 12 and 13) are characterized by a gradual decrease in staining capacity of the larger granules, simultaneously with a continued increase of the inter-

vening fibrillar bridges, until the nuclear wall becomes visible and presents a homogeneous appearance (Figs. 15, 16, etc.). This membrane, though invisible prior to this time, remains very conspicuous until the prophase of the first spermatocyte division. *These observations, while not conclusive, indicate that the nuclear membrane may be, at least in part, chromatic.*

LITERATURE CITED.

Boring, Alice M.

'07 A Study of the Spermatogenesis of Twenty-two Species of the Membracidæ, Jassidæ, Cercopidæ and Fulgoridæ, with Special Reference to the Behavior of the Odd Chromosome. Jour. Exp. Zool., Vol. 4.

Carnoy, J. B.

'85 La Cytodiérése chez les Arthropodes. La Cellule, T. I.

Dederer, Pauline H.

'07 Spermatogenesis in Philosamia cynthia. BIOL. BULL., Vol. 13.

Eisen, Gustav.

'00 Spermatogenesis of Batrachoseps. Jour. Morph., Vol. 17.

King, H. D.

'08 The Oögenesis of Bufo lentiginosus. Jour. Morph., Vol. 19.

McClendon, J. F.

'07 The Spermatogenesis of Pandarus sinnatus Say. BIOL. BULL., Vol. 13.

Medes, Grace.

'04 The Spermatogenesis of Scutigera forceps. BIOL. BULL., Vol. 9.

Montgomery, Thos. H.

'98 Comparative Cytological Studies, with Especial Regard to the Morphology of the Nucleolus. Jour. Morph., Vol. 15.

Payne, Fernandus.

'09 Some New Types of Chromosome Distribution and their Relation to Sex. BIOL. BULL., Vol. 16.

'12 A Further Study of the Chromosomes of the Reduviidæ. Jour. Morph., Vol. 23.

Randolph, Harriet.

'08 The Spermatogenesis of the Ear-wig, Anisolabis martima. BIOL. BULL., Vol. 15.

Ruzicka, Vladislav.

'06 Struktur und Plasma. Ergebnisse der Anatomie und Entwickelungegeschichte, Band 16.

Stevens, N. M.

'08 A Study of the Germ Cells of Certain Diptera, with Reference to the Hetero-chromosomes and the Phenomena of Synapsis. Jour. Exp. Zool., Vol. 5.

'09 Further Studies on the Chromosomes of the Coleoptera. Jour. Exp. Zool., Vol. 6.

'10 The Chromosomes in the Germ Cells of Culex. Jour. Exp. Zool., Vol. 8.

'11 Hetero-chromosomes in the Guinea Pig. BIOL. BULL., Vol. 21.

Wilson, E. B.
'12 Studies on Chromosomes. Observations on the Maturation Phenomena in Certain Hemiptera and Other Forms, with Consideration on Synapsis and Reduction. Jour. Exp. Zool., Vol. 13.
'05 Studies on Chromosomes. The Behavior of the Idiochromosomes in Hemiptera. Jour. Exp. Zool., Vol. 2.

EXPLANATION OF PLATES.

PLATE I.

FIG. 1. Contraction stage with chromatic fibers bearing granular enlargements. The nucleolus is shown in this, and the three succeeding figures as a homogeneous body.

FIGS. 2 AND 3. Chromatin threads emerging from the contraction stage.

FIGS. 4 AND 5. Further elongation of the chromatin threads. The enlargements have become smaller but more compact.

FIGS. 4 TO 17. Successive stages in the formation of the nucleolus.

FIGS. 6 AND 7. Individualizing the granules found in succeeding stages. Nucleolus shows its achromatic nature.

FIGS. 8 TO 13. Stages in the formation of the nuclear wall. Further differentiation of the chromatin and achromatin.

FIG. 14. Various shapes assumed by the chromatic and achromatic substance of the nucleoli.

FIGS. 15 AND 16. Nuclei showing further development of the nucleolus.

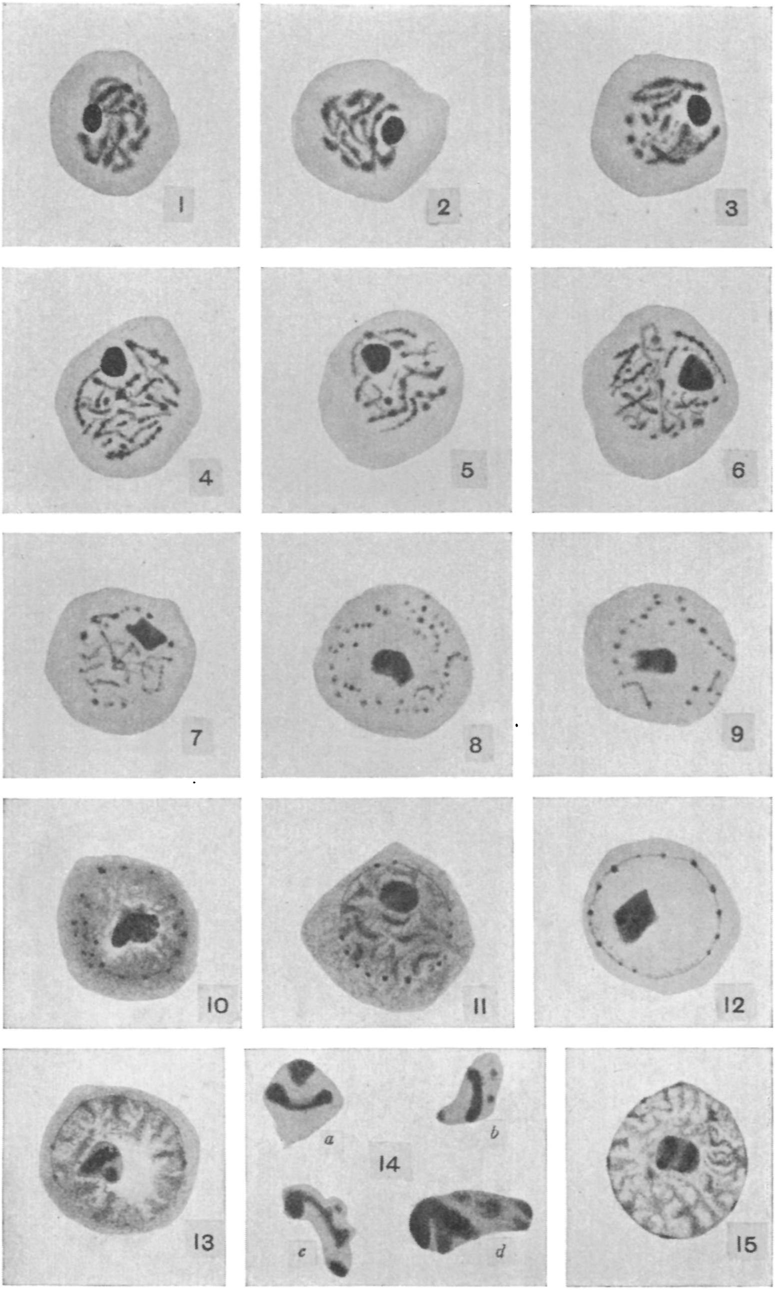

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PLATE II.

FIGS. 17 TO 20. Nuclei with nucleoli made up of achromatin and the sex-chromosomes. Linin approaching the nucleoli.

FIGS. 20 TO 29. Successive stages in the disappearance of the nucleolus.

FIG. 21. Fusion of achromatic part of the nucleolus and linin.

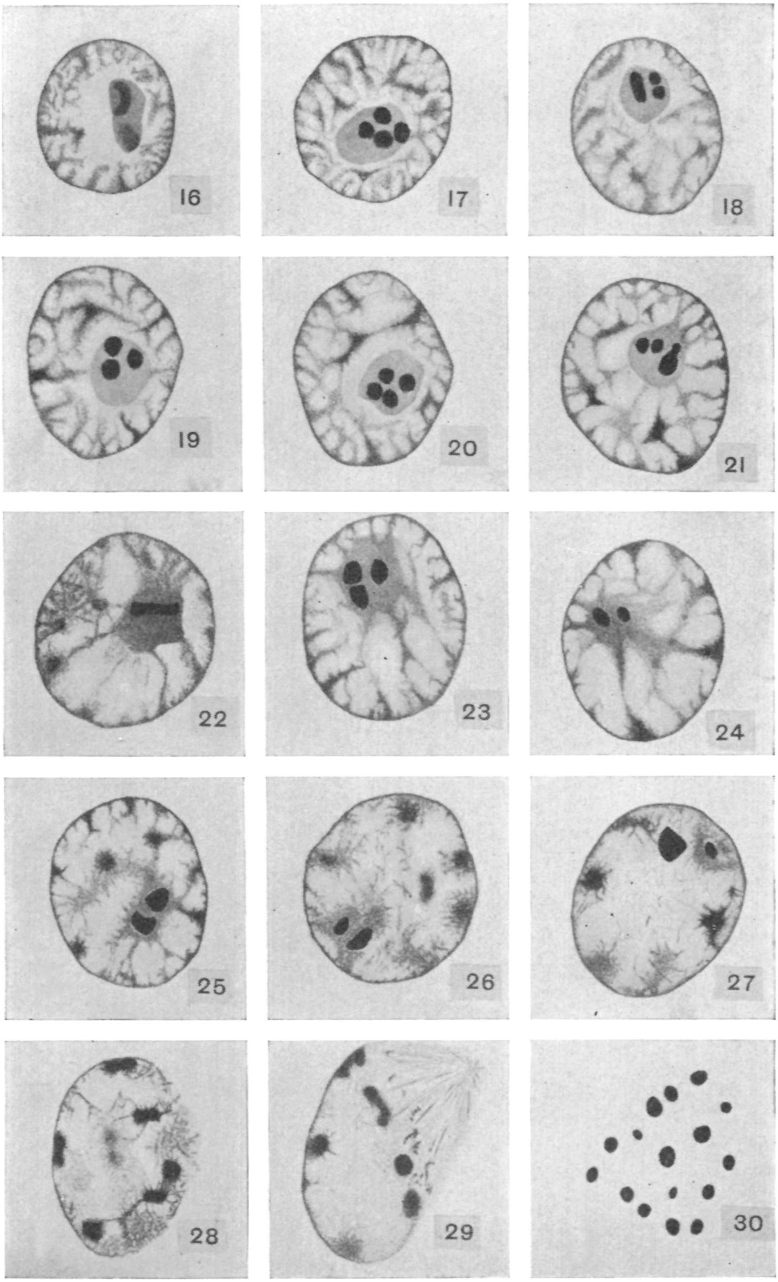
FIGS. 22 TO 25. Successive stages in the fusion of the true nucleolus and linin, the former becoming indistinguishable from the latter.

FIGS. 25 TO 30. Formation of the prophase chromosomes for the first spermatocyte division.

FIGS. 26 AND 27. Remains of the old nucleolus being drawn to the nuclear membrane.

FIGS. 28 AND 29. Breaking down the nuclear membrane by the formation of the first spermatocyte spindle.

FIG. 30. Metaphase plate of the first spermatocyte division.



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